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☐ 1: Mol Cell Endocrinol. 2002 Mar 28;189(1-2):191-9.

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**Characterization of promoter 1B in the human glucocorticoid receptor gene.****Nunez BS, Vedeckis WV.**

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At least three different promoter regions (1A, 1B, and 1C) are involved in the expression of the human GR gene. Promoters 1B and 1C are found in a 2800 bp region of DNA immediately upstream of the exon 1C transcriptional initiation site. Transcripts containing either exon 1B or 1C are expressed in a wide variety of human tissues and cultured cells. Luciferase reporter constructs were created containing promoter 1B plus 1C (-2738 to +19), promoter 1B (-2738 to -1046) alone, or promoter 1C (-1045 to +19) alone. All three constructs were equally effective in driving luciferase expression in HeLa (human cervical carcinoma) cells. In Jurkat (human T-cell acute lymphoblastic leukemia) cells, constructs containing promoters 1B plus 1C or promoter 1B were equally active, but the promoters 1B plus 1C construct was 35% more active than the promoter 1C construct. However, in HepG2 (human hepatoma) cells, the promoter 1C construct was as effective as promoters 1B plus 1C and more than twice as effective as promoter 1B. Sequences that reside proximal to the exon 1B transcriptional start site included three Sp1 (FP2-FP4) sites. Another site (FP1) contains the sequence TGATAG, which strongly resembles the consensus binding sequence for the GATA family of transcription factors. However, oligonucleotide competition and supershift analysis of FP1 indicates that this site is not a binding site for GATA proteins. These four sites are in addition to three YY1 and one Sp1 sites previously reported in promoter 1B. In HeLa cells, deletion of the three YY1 sites results in only a 30% loss of activity and substantial loss of activity occurs only after deletion of all four Sp1 sites, indicating the critical importance of Sp1 in GR expression in these cells. In contrast, the elimination of the three YY1 sites results in a dramatic decrease in promoter strength in both HepG2 and Jurkat cells (64 and 77%, respectively), while subsequent deletions of promoter elements do not result in substantial changes in promoter activity in these cell lines. This study shows that both promoters 1B and 1C are important for the ubiquitous expression of the

human GR gene. Differences in the utilization of these promoters in various cell types are likely a reflection of different promoter availability and/or the levels of specific transcription factors in the cell. This could contribute to tissue-specific expression of GR levels in different cell types.

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